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NATIONAL STANDARD OF
THE PEOPLE'S REPUBLIC OF CHINA

GB 5009.128-2016

National food safety standard - Determination of cholesterol in foods

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Foreword

This Standard replaces GB/T 5009.128-2003 *Determination of cholesterol in foods*, GB/T 22220-2008 *Determination of cholesterol in foods - High-performance liquid chromatography* and GB/T 9695.24-2008 *Meat and meat products - Determination of cholesterol*.

Compared with GB/T 5009.128-2003, the main changes in this Standard are as follows:

- modified the standard's name to "National food safety standard - Determination of cholesterol in foods";
- added gas chromatography as method one and high-performance liquid chromatography as method two; modified colorimetric method as method three;
- modified extraction solvent, the amount of ethanol and volume constant volume in pre-treatment method of gas chromatography in GB/T 9695.24-2008.

National food safety standard -

Determination of cholesterol in foods

1 Scope

This Standard specifies the determination methods of cholesterol in foods.

This Standard applies to the determination of cholesterol in foods. Method One Gas chromatography is applicable to the determination of cholesterol in meat and meat products, eggs and egg products, milk and dairy products and other animal foods and vegetable oils and fats. Method Two High performance liquid chromatography is applicable to the determination of cholesterol in meat and meat products, eggs and egg products, milk and dairy products and other animal foods. Method Three Colorimetric method is applicable to the determination of cholesterol in meat and meat products, eggs and egg products and other animal foods.

Method One -- Gas chromatography

2 Principle

Saponify the sample by absolute ethanol - potassium hydroxide solution. Extract by petroleum ether and anhydrous ether mixture. Concentrate the extract to dry. After dissolving and setting volume of absolute ethanol, use gas chromatography to detect, external standard method to quantify.

3 Reagents and materials

Unless otherwise indicated, the reagents used in this method are of analytical grade, water is grade one water specified in GB/T 6682.

3.1 Reagents

3.1.1 methanol (CH_3OH): chromatographic pure

3.1.2 anhydrous ethanol ($\text{C}_2\text{H}_5\text{OH}$)

3.1.3 petroleum ether: boiling range $30^\circ\text{C} \sim 60^\circ\text{C}$

3.1.4 anhydrous ether ($\text{C}_4\text{H}_{10}\text{O}$).

Take 200 g of edible part of sample to homogenize. Place the sample in a sealed container to prevent deterioration and composition changes. The sample shall be analyzed as soon as possible within 24 hours of homogenization.

5.1.2 Vegetable oil, dairy products and other liquid samples

Take the uniform liquid sample after mixing into a sealed container to be tested.

5.2 Sample processing

5.2.1 Saponification

Weigh 0.25g ~ 10g of prepared sample (nearest to 0.001 g, cholesterol content of about 0.5mg ~ 5mg) in a 250-mL round-bottomed flask. Add 30 mL of absolute ethanol, 10 mL of 60% potassium hydroxide solution. Well mix. Heat the sample at 100°C magnetic stirring heater for saponification reflux 1h. Shake from time to time so as to prevent sample sticking to the bottle wall. After the saponification ends, use 5 mL of absolute ethanol to rinse its interior from the condenser top. Remove the round-bottomed flask. Cool to room temperature with running water.

5.2.2 Extraction

Quantitatively transfer of all saponified liquid in a 250-mL separatory funnel. Use 30 mL of water to rinse the round-bottomed flask in 2~3 times. Merge the washing liquid into the separatory funnel. Then use 40 mL of petroleum ether - anhydrous ether mixture (1+1, volume ratio) to rinse the round-bottomed flask in 2~3 times. Then merge into the separatory funnel. Shake 2 min, place still and stratify. Transfer the water phase. Combine the three organic phases. Wash the extract to neutral with 100 mL of water each time. Swirl gently for the first time to prevent emulsification. The extract shall be dehydrated with about 10 g of anhydrous sodium sulfate to a 150-mL flask.

5.2.3 Concentration

Evaporate the extract in the flat-bottomed flask to near dryness under vacuum condition. Use absolute ethanol to dissolve and set volume to 5 mL. Determine with gas chromatograph.

The pretreatment of different sample requires blank test at the same time.

5.3 Determination

5.3.1 Instrument reference conditions

- a) Chromatographic column: DB-5 flexible quartz capillary column, column length of 30 m, inner diameter of 0.32 mm, particle size of 0.25 μ m, or

Take 200 g of edible part of sample. Use a meat grinder or homogenizer to homogenize the sample. Place the sample in a sealed container to prevent deterioration and composition changes. The sample shall be analyzed as soon as possible within 24 hours of homogenization

12.1.2 Dairy and other liquid samples

Take well mixed uniform liquid sample into a sealed container for testing.

12.2 Sample processing

12.2.1 Saponification

Weigh 0.25g ~ 10g of prepared sample (nearest to 0.001 g, cholesterol content of about 0.5mg ~ 5mg) in a 250-mL round-bottomed flask. Add 30 mL of absolute ethanol, 10 mL of 60% potassium hydroxide solution. Well mix. Heat the sample at 100°C magnetic stirring heater for saponification reflux 1h. Shake from time to time so as to prevent sample sticking to the bottle wall. After the saponification ends, use 5 mL of absolute ethanol to rinse its interior from the condenser top. Remove the round-bottomed flask. Cool to room temperature with running water.

12.2.2 Extraction

Quantitatively transfer of all saponified liquid in a 250-mL separatory funnel. Use 30 mL of water to rinse the round-bottomed flask in 2~3 times. Merge the washing liquid into the separatory funnel. Then use 40 mL of petroleum ether - anhydrous ether mixture (1+1, volume ratio) to rinse the round-bottomed flask in 2~3 times. Then merge into the separatory funnel. Shake 2 min, place still and stratify. Transfer the water phase. Combine the three organic phases. Wash the extract to neutral with 100 mL of water each time. Swirl gently for the first time of water washing to prevent emulsification. The extract shall be dehydrated with about 10 g of anhydrous sodium sulfate to a 150-mL flask.

12.2.3 Concentration

Evaporate the extract in the flat-bottomed flask to near dryness under vacuum condition. Use absolute ethanol to dissolve and set volume to 5 mL. The solution passes through a 0.45 µm filter membrane. Collect the filtrate in the sample bottle. Determine with high performance liquid chromatograph.

The pretreatment of different sample requires blank test at the same time.

12.3 Determination

12.3.1 Instrument reference conditions

- a) Chromatographic column: C18 reversed-phase column, column length

17.4.2 Cholesterol standard working solution (100 µg/mL): pipette 10 mL of cholesterol standard stock solution (1.0 mg/mL); use glacial acetic acid to dissolve and set volume to 100 mL. Prepare when using.

18 Instruments and equipment

18.1 Homogenizer

18.2 Spectrophotometer

18.3 Electronic balance: divisions of 1 mg and 0.1 mg

19 Analysis steps

19.1 Production of cholesterol standard curve

Pipette 0.0 mL, 0.5 mL, 1.0 mL, 1.5 mL, 2.0 mL of cholesterol standard working solutions respectively in 10 mL tubes. Add glacial acetic acid into each tube to make the total volume reach 4 mL. Along with the tube wall, add into 2 mL of iron alum color solution; well mix. Within 15min ~ 90min, at wavelengths of 560nm ~ 575nm, perform colorimetric. Using cholesterol standard concentration as abscissa, the absorbance as ordinate, make the standard curve.

19.2 Determination

19.2.1 Extraction and determination of fat in foods

Based on food categories, it shall use Soxhlet fat extraction method, grinding extraction method and Roche method to extract fat, respectively. And calculate the fat content per 100 g of food.

19.2.2 Determination of cholesterol in foods

Place 3~4 drops of extracted oil fats (cholesterol content of about 300 µg ~ 500 µg) into a 25-mL tube. Accurately record its mass. Add into 4 mL of anhydrous ethanol, 0.5 mL of 50% potassium hydroxide solution. Well mix. Equip with condenser. Carry out saponification in 65°C constant temperature water bath for 1h. During saponification, shake once every 20min ~ 30min to make a complete saponification. After the saponification ends, cool with running water. Add into 3 mL of 5% sodium chloride solution, 10 mL of petroleum ether. Fasten the glass stopper. Shake 2min on the electric shaker. Place still for stratification (usually taking about 1h or more).

Take 2 mL of upper petroleum ether solution. Place in a 10mL plugged glass tube. Dry with nitrogen in a 65°C water bath. Add into 4 mL of glacial acetic acid,

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